CYTRIX Cell Encapsulation Protocol

Microfluidic Production of Cell-Laden Hydrogel Microbeads

Product Description

Microfluidic cell encapsulation is commonly carried out in liquid w/o emulsions. The CYTRIX hydrogel kit allows encapsulation of cells in 3D hydrogel microbeads to more accurately represent physiological microenvironments. The hydrogels are permeable to oxygen and nutrients and offer the possibility to conduct longitudinal studies on encapsulated cells. Additionally, CYTRIX enables the co-encapsulation of multiple cell types in micro confinements to study cell-cell interactions.

In contrast to existing hydrogel encapsulation methods, CYTRIX is exceptionally cell-friendly, easy-to-use, and does not require specialised hardware. While allowing hour-long encapsulation without clogging microfluidic channels, the method does not require any non-physiological gelation triggers such as pH, UV light, radical chemistry, or temperature changes.

Kit Specification

Item	Description	Use
CYTRIXBio CYTRIX A & B solutions	2 x 800 uL buffered pregel solutions pH 7.0 Cat.No. C800	Formation of biocompatible hydrogel microbeads
Sphere Fluidics Pico-Gen™ 60x60 [included in the bundle]	Double-aqueous microfluidic picodroplet generator Cat.No. C112	Microfluidic picodroplet formation
Sphere Fluidics Pico-Surf™ (2% (w/w) in Novec™ 7500) [included in the bundle]	Fluorinated oil with fluorosurfactant as biocompatible carrier phase Cat.No. C021	Picodroplet emulsification

Storage and Stability

The CYTRIX solutions are stable for a minimum of 6 months after manufacturing if stored at 2 - 8°C.

Precautions and Limitations

- For Research Use Only. Not for use in diagnostic procedures.
- The physical, chemical, and toxicological properties of these products may not yet have been fully investigated; therefore, we recommend the use of gloves, lab coats, and eye protection while using these chemical reagents.

Required Materials not Provided in This Kit

Equipment

Item	Description	Use
Syringe pumps or pressure-driven pumping system	Suited for flow rates of 250 - 1000 uL/h	
Micropipettes	Suited for volumes of 200 - 1000 uL	
Centrifuge		Washing of beads
Vortex		Washing of beads

Reagents

Item	Description	Use
Cell line(s) of interest		
Cell culture medium		
High fluorinated oil	E.g. Sphere Fluidics Pico-Wave™ 7500 Cat.No. C094, C095, C096	Breaking emulsions after collection
Emulsion breaking solution	E.g. Sphere Fluidics Pico-Break™ Cat.No. C081, C082	Breaking emulsions after collection

Disposables

Item	Description	Use
[Optional] Cell strainer	E.g. pluriSelect™ pluriStrainer™ 40 um Cat.No. 43-10040-XX	Obtaining uniform single-cell suspension
2 x 1 mL syringe	E.g. BD Luer-Lok™ 1 mL syringe Cat.No. 309628	Injection of aqueous solutions
1 x 5 mL syringe	E.g. BD Luer-Lok™ 5 mL syringe Cat.No. 309646	Injection of carrier fluid
Microfluidic tubing & connectors	E.g. PE tubing (OD 1.09 mm, ID 0.38 mm) with Luer Lock adaptor & silicone sleeve OR PE tubing (OD 1.32 mm, ID 0.86 mm) with blunt 20 gauge cannulas	Interfacing
Collection vials suitable for centrifugation	E.g. 2 and 5 mL Eppendorf™ tubes	Collection & washing of beads
Pipette tips	Suited for volumes of 200 - 1000 uL	

Microfluidic Encapsulation Protocol

1. Sample Preparation

- **Preparation of CYTRIX solutions**
  - Prepare a concentrated cell suspension in cell culture medium of choice (recommended cell count: 15 million/mL to yield a cell concentration of 3 million cells/mL final CYTRIX solution).
  - [Optional] For adherent cell types: strain cell suspension through cell strainer of appropriate mesh size to obtain uniform single-cell suspension.
  - Add 200 uL of the cell suspension to each vial containing 800 uL CYTRIX pre-gel solution. Mix thoroughly by pipetting up and down several times.
  - Draw out each of the cell-containing CYTRIX solutions into separate 1 mL syringes.
- **Preparation of carrier fluid**
  - Use the provided 2% (w/w) Pico-Surf™ in Novec™ solution or prepare a carrier fluid of 2 % (w/w) Pico-Surf™ in Pico-Wave™ 7500.
  - Draw out the carrier fluid into a 5 mL syringe.

NB! To maximize the yield of hydrogel beads containing single cells with the Pico-Gen™ 60 X 60 picodroplet generator, each CYTRIX solution should contain 3 million cells/mL.

2. Microfluidic Encapsulation

- **Setting up the microfluidic experiment**
  - Mount all syringes on individual syringe pumps (2 x 1mL syringe with CYTRIX solutions, 1 x 5mL syringe with carrier fluid) or use your pressure-driven system.
  - Connect the syringes with the Pico-Gen™ picodroplet generator using your microfluidic connectors & tubing of choice.
  - At the outlet channel, insert tubing connected to a waste collection vial.
  - Start by injecting the carrier fluid at a flow rate of 800 uL/hr.
  - Once all channels are filled with carrier fluid, start injecting both CYTRIX solutions simultaneously at a flow rate of 250 uL/hr each.
  - Wait until flow and picodroplet formation are stable. Once stable, replace the waste collection vial with a clean collection vial containing 2 mL cell culture medium (e.g. 5 mL Eppendorf™ tube).
  - Collect for 1 - 2 hours.

NB! It is critical that the precursor solutions do not mix prior to the junction where all three fluids meet. If backflow occurs, try temporarily increasing flow rates in the affected channels.

3. Washing

- **Break emulsions**
    - Add Pico-Wave™ 7500 with approx. 20% Pico-Break™ to the collected sample (approx. equal amount of collected sample volume).
    - Vortex for approx. 10 - 15 s.
    - Centrifuge at 1000 rcf for 1 min to separate carrier fluid, hydrogel beads, and excess cell culture medium.
    - Carefully remove carrier fluid from the bottom of the sample using a pipette.
- **Wash with buffer/cell culture medium**
    - Add buffer/cell culture medium (approx. 2x sample volume).
    - Vortex for approx. 10 - 15 s.
    - Centrifuge at 1000 rcf for 1 min.
    - Carefully remove remaining carrier fluid from the bottom of the sample (*NB! Submerge pipette all the way down and remove oil that is visible to the naked eye together with the cloudy phase at the interface, which consists of oil-water droplets; Hydrogel beads are dispersed in the aqueous phase and are not visible to the eye*).
    - Optionally repeat the previous four steps.

NB! Using a bright-field microscope, it can be difficult to see the hydrogel beads in aqueous suspension. We recommend using phase contrast or minimising the size of the condenser aåerture diaphragm for maximal contrast.